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Effect of long term storage of sugarcane spirit in stainless steel on physiochemical

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Abstract

Cachaça and sugarcane spirits are beverages consumed in Brazil and all over the world. These beverages are usually stored in wooden or stainless steel containers. The objectives of this work were to analyze the physicochemical and chromatographic profile of sugarcane spirits produced in different harvests and the influence on the quality parameters of this beverage stored in stainless steel containers. Fifteen samples from crops produced between 1996 and 2016 and stored in stainless steel tanks were analyzed. There was a wide variation in the percentages of secondary compounds and contaminants. Of the 15 samples analyzed, the compositions of five samples were outside the legal limits: A/1996 (butan-1-ol concentration was above 3.0 mg/100 mL of anhydrous alcohol (aa)), D/2000 (concentration of alcohol and congeners was lower than 38% v/v and 200 mg/100 mL of aa, respectively), F/2002 (concentration of congeners were lower than 200 mg/100 mL of aa), G/2003 (butan-1-ol concentration was greater than 3.0 mg /100 mL of aa), N1/2016 (aldehyde concentrations were greater than 30 mg/100 mL of aa), Thus, these samples are not recommended for consumption and commercialization. None of the samples contained copper concentratons that exceeded the legal limit. Zinc was detected at concentrations lower than 1.0 mg/L. Cadmium, chromium, lead and iron were not detected. The storage of the beverage in stainless steel barrels for a period of up to 20 years modified the physical-chemical characteristics of the spirit. However, it does not offer risks regarding the contamination of the product by metals because, when detected, they were present in concentrations that that would not be harmful for consumers.

Keywords: Storage, Contaminants, Heavy metals, Quality, Distilled.
Abbreviations: Ministry of Agriculture, Livestock and Supply (MAPA)

Introduction

Cachaça is a genuinely Brazilian fermented and distilled beverage, which can be aged in wooden barrels or stored in stainless steel barrels. Its consumption in the national territory is related to historical and cultural reasons, considering that the appearance of the beverage coincides with the colonization process in Brazil. In addition, this beverage has pleasant sensory characteristics and is available in different price ranges and commercial establishments, being accessible to all people of legal age from all social classes (Cardoso, 2020).

In Brazil, there are 951 cachaça-producing establishments registered with the Ministry of Agriculture, Livestock and Supply (MAPA). These establishments are present in practically all the states. Of these registered establishments, 421 are located in the state of Minas Gerais, which characterizes the state as one of the main producers of cachaça and sugarcane spirits produced mainly in copper stills (Brasil, 2019). In view of the dimension of the production chain, the sector shows great potential for the expansion of its exports through the inclusion of new establishments, products and brands, but mainly through the valorization of the product through investment in production and storage techniques that maintain product quality (Paiva et al., 2017).

The storage of cachaça or cane spirits in stainless steel containers configures a maturation process, or a "softening", in which the components of the cachaça naturally react with each other, such as alcohols and acids that react to form esters, making it more pleasing to the palate. Unlike the aging process, where more than 300 chemical reactions can occur, the contact of the beverage with the material in the tanks during the storage period in stainless steel barrels should not result in interactions and incorporation of compounds from the storage container. The chemical and sensory quality of the spirit produced should be preserved (Alcarde et al., 2010).

Stainless steel tanks are widely used for storage, transport and processing of beverages because of their resistance to corrosion and good mechanical properties. The most widely used are the austenitic tanks, which are composed of at least 16% chromium and 6% nickel in addition to iron. Other alloying elements can also be incorporated into the structure, such as manganese, molybdenum, and niobium, among others (Almomani et al., 2019; Zuñiga-Diaz et al., 2020). However, the care, form and time of use and the characteristics of the product to be stored in stainless steel tanks influence its properties and durability. Changes in its structure, such as corrosion, dissolution and friction, can result, changing the characteristics of the inert material and enabling the interaction with the stored beverage. Thus, changes in the physicochemical characteristics of the beverage and contamination of the product by metals or metal complexes that are formed can occur, representing a risk to consumers (Ibanez et al., 2008; Fernández-López et al., 2018; Atapour et al., 2020; Zuñiga-Diaz et al., 2020).

When stainless steel tanks are ready for use, the storage of the beverage in these containers must preserve its physicochemical profile and sensory characteristics. These compounds are extremely important for the composition of the flavour of the cachaça, and they are defined and regulated by Normative Instruction no. 13, of 2005 of MAPA. Esters, aldehydes, alcohols, and organic acids are among the congeners. In addition to congeners, the legislation determines the maximum levels of contaminants that can be present in the distillate, such as methanol, ethyl carbamate, copper and lead, among others. Using the IQS's (Identity and Quality Standards), the quality and safety of the beverage produced can be ensured, these being fundamental for the health of the consumer (Brasil, 2005a).

Studies on the storage of cachaça in wooden containers are common, but the evaluation of storage in stainless steel containers for long periods of time has not yet been performed. Thus, the objective of this study was to evaluate the effects of storing sugarcane spirits in stainless steel barrels for a period of 20 years on the physicochemical quality and presence of contaminants in the beverage.

Results and Discussion

Physicochemical characteristics

The physicochemical characteristics of the sugarcane spirit samples stored in stainless steel vats from 1996 to 2016 are shown in **Table 1**. shows that samples differed from one another in terms of alcohol content (ethanol), which ranged from 36.68% (v/v) in sample D from the year 2000 to 51.38% (v/v) in sample N2 from 2016, with sample D having an alcoholic strength below the minimum legal limit. In general, the lowest alcoholic strengths were observed in samples with longer storage times, produced until the year 2003. The reduction in the alcohol level might have occurred because of the formation of esters, oxidation, partial evaporation of alcohol due to the presence of an empty space inside the tank or all three. In addition, the samples were produced from different crops, and, despite being from the same agro-industrial system and the maximum efforts being employed to maintain the same standards, differences in their characteristics can occur.

The concentration of dry extract in the samples ranged from 0.1 g/L (N1 and N2/2016) to 1.37 g/L (A/1996). Higher quantities of dry extract were observed for samples stored for longer periods. The average value was 0.70 g/L in samples produced up to 2003, a longer storage time, whereas the mean content was 0.13 g/L in samples produced from 2008, a shorter storage time. The low concentrations of dry extract found were already expected because sucrose was not added to the beverage, nor did it have contact with wooden containers, which could lead to an accumulation of soluble solids in the distillate.

The volatile acidity exerts a great influence on the sensory perception of the beverage; in addition, the presence of adequate amounts of acidic compounds is of great importance for the quality of the beverage because the acids react with the alcohols present during its production to increase the formation of esters, which are of high importance for the sensory characteristics of the beverage (Cardoso, 2020). The samples analyzed differed statistically, and the acidity values varied from 19.19±1.71 to 143.69±0.16 mg/100 mL of anhydrous alcohol (samples D/2000 and B/1998, respectively). The maximum legal limit for this parameter is 150 mg/100 mL of anhydrous alcohol. A high acidity can be related to the fermentation process, where the aeration of the must can favor acetic fermentation. The increase in the concentration of this compound can also be related to the storage of the beverage, where the oxidation of ethanol to acetaldehyde, and then to acetic acid, an occur (Bortoletto et al., 2016; Cardoso, 2020).

Sample A/1996 had the highest concentration (5.03±0.03 mg/100 mL of anhydrous alcohol) of furfural and 5-hydroxymethylfurfural, but it was still within the maximum allowed limit of 5.0 mg/100 ml of aa. The lowest concentration was observed in sample L/2014 (0.52 ± 0.01 mg/100 mL of aa). The high concentrations of furfural in the distillate are related to the use of burnt cane for production and the use of a high temperature in the distillation stage. Furfural results from the chemical decomposition of carbohydrates, and it can be formed at different stages of the cachaça production process, such as the pyrolysis of organic matter deposited at the bottom of stills or even during the aging of the beverage through the action of acids on pentoses and their polymers (hemicelluloses), which are present in wooden containers used in storage. Because of this fact, furfural and 5-hydroxymethylfurfural can be found in higher concentrations in cachaça stored in wooden barrels (Azevedo et al., 2007; Masson et al., 2007; Zacaroni et al., 2011; Bortoletto et al., 2016). Thus, storage in stainless steel vats, even for long periods, avoids the increase in the concentration of these undesirable compounds in the beverage.

The concentrations of aldehydes in the spirits ranged from 3.91±0.25 (sample D/2000) to 39.67±0.70 mg/100 mL of anhydrous alcohol (sample N1/2016), this being the only sample that exceeded the maximum legal limit of 30.0 mg/100 mL of aa. Aldehydes are characteristic of the head fraction because they are highly volatile (Alcarde et al., 2010; Cardoso, 2020). Therefore, concentrations greater than legal limit can be associated with the errors due to separation of fractions during distillation. The oxidation of aldehydes results in the formation of organic acids, and the reaction with alcohol forms acetals. The balance between acetals and aldehydes is particularly important for a specific aroma because aldehydes often have an unpleasant and pungent odor, whereas the aroma of acetals is pleasant and fruity (Piggot and Conner, 2003; Miranda et al., 2008).

Ester concentrations ranged from 6.19±0.81 (sample D/2000) to 75.96±2.51 mg/100 mL of aa (sample J/2011). Esters are a class of volatile compounds that are very important for the taste and aroma of the beverage, and their legal limit is 200.0 mg/100 mL of anhydrous alcohol. Esters are formed through the reaction between alcohols and organic acids present in the beverage. Ethyl acetate is the principal component of this group of substances, and it is responsible for the pleasant aroma of beverages. In addition, some esters, especially ethyl lactate, are linked to bacterial contamination of the must, such as by *Lactobacillus spp*. (Faria et al., 2003; Parazzi et al., 2008; Hirst and Richter, 2016; Walker and Stewart, 2016). Even after storage for several years in stainless steel vats, high concentrations of esters were not observed. Higher concentrations of esters can be found when beverages are aged in wooden containers. Santiago et al. (2014) found concentrations of 51.24 to 101.03 mg/100 mL anhydrous alcohol for esters in cachaça stored in amburana barrels, resulting from the reactions of alcohol with wood components.

The higher alcohol content in sugarcane spirits comes from the sum of propanol, 2-methylpropanol and 3-methylbutanol concentrations. The concentrations observed ranged from 131.24±0.45 (sample B/1998) to 264.79±2.96 mg/100 mL of anhydrous alcohol (sample N1/2016). The highest concentrations of higher alcohols were observed in beverages stored for a shorter period (2014, 2015 and 2016). No sample approached the maximum legal limit of 360 mg/100 mL of anhydrous alcohol. High concentrations of higher alcohols can be related to an efficiently conducted distillation process, where the correct separation of the head, heart and tail fractions occurs because these fractions contain different concentrations of these alcohols (Silva et al., 2020). However, the lower concentrations of higher alcohols observed for beverages stored for longer periods indicate that reactions that resulted in the degradation of these compounds might have occurred, resulting in changes in the composition of the cachaça.

The congeners represent the sum of the concentrations of volatile acidity, esters, aldehydes, furfural and higher alcohols. According to Brazil (2005a), the total concentration must lie between the minimum limit of 200 mg/100 mL of aa and the maximum limit of 650 mg/100 mL of aa These compounds are responsible for the aroma and taste of the beverage, composing the so-called “flavour” or “bouquet” of sugarcane spirits (Pereira et al., 2003; Alcarde et al., 2010; Cardoso, 2020). Samples D/2000 and F/2002 did not contain the minimum necessary concentration of congeners; They contained 198.49±5.76 and 193.18±1.42 mg/100 mL of aa, respectively. Sample D/2000 did not contain the minimum concentration of ethanol, and it contained the lowest concentrations of aldehydes, esters and volatile acidity of all the samples. The sample that contained the highest concentration of congeners was sample N1/2016 (437.44±2.43 mg/100 mL of aa), but it was still within the maximum limit of 650 mg/100 mL of aa.

Glycerol was not detected in the samples within the working range used. This fact suggests that the compounds naturally present in sugarcane spirit do not react with one another to form glycerol, so this compound does not appear in large amounts even after storage in stainless steel containers for a long period of time. Pure distillates typically exhibit low concentrations of glycerol because of its high boiling point (290 °C). On the other hand, when the beverage is stored in wooden barrels, higher concentrations of glycerol can be found. It is formed by the transesterification of wood triglycerides with ethanol to produce fatty acid ethyl esters and free glycerol during the maturation and aging process (Lee et al., 2001; Bortoletto et al., 2016).

Organic contaminants

The levels of contaminants present in sugarcane spirits stored in stainless steel vats from 1996 to 2016 are presented in **Table 2**. In addition to the higher alcohols that contribute to the quality of the beverage, some higher alcohols are classified as contaminants. This is the case of butan-1-ol and butan-2-ol. The maximum legal limit of butan-1-ol in sugarcane spirits is 3.0 mg/100 mL of aa, a value that was exceeded by 35.67% in sample A (1996), and by 18% in sample G (2003), which presented mean concentrations of 4.07±0.08 and 3.54±0.05 mg/100 mL of aa, respectively. The samples 2011 to 2016 contained the lowest concentrations of butan-1-ol, with no significant differences. The maximum legal limit of butan-2-ol is 10 mg/100 mL of aa, and the concentrations found in the samples ranged from not detected (L and H samples) to 4.76±0.02 mg/100 mL of anhydrous alcohol (sample N1). These two alcohols can be formed by acetobutyl bacteria, which, during the cane storage process and before the crushing process, can contaminate the raw material and be present in the fermentation broth. Furthermore, amino acid transformations during the fermentation process also result in greater formation of higher alcohols because of the low yeast activity, the presence of non-*Saccharomyces* yeasts, the high temperatures and the low pH of the must (Swiegers et al., 2005; Walker and Stewart, 2016; Cardoso, 2020).

Methanol is another alcohol that is considered to be a contaminant. The maximum legal limit established for methanol is 20 mg/100 mL of anhydrous alcohol. All the samples contained concentrations below this limit, with values ranging from not detected (2016 samples) to 2.86±0.16 mg/100 mL of anhydrous alcohol (sample D/2000). This result demonstrates that there was an effective treatment of the sugarcane juice with filters and decanters to remove the bagasse (rich in pectin) and other impurities that could contribute to the formation of this contaminant during fermentation. The bagasse is rich in pectic substances, which are polymers of galacturonic acid with a variable degree of methoxylation. The action of yeast pectic enzymes releases methanol. In the body, methanol is oxidized to formic acid and then to CO_2, causing acidosis (decrease in blood pH) and affecting the respiratory system, which can lead to coma and even death (Cardoso, 2020).

Another organic contaminant that can be present in sugarcane spirits is ethyl carbamate (EC). The highest concentration of ethyl carbamate was detected in sample A/1996 (51.47±0.93 $\mu g\ L^{-1}$), the oldest sample, and the lowest concentration was found in samples N1 and N2 (below the LQ and 9.24± 0.06 $\mu g\ L^{-1}$, respectively), both from the year 2016. In all the samples, the EC concentrations remained considerably below the maximum limit of 210 $\mu g\ L^{-1}$ allowed by Brazilian legislation for cachaça (Brasil, 2014). Mendonça et al. (2016) found mean EC concentrations of 9.89 $\mu g\ L^{-1}$ in the heart fractions of freshly distilled cachaça produced by wild yeasts in the presence of rice bran and 9.84 $\mu g\ L^{-1}$ in the beverage produced by wild yeasts in the presence of a mixture of corn bran and rice bran. The authors also investigated the EC concentration during the storage of the heart fraction. The only beverage in which EC was not quantified during the six months of storage was that produced with corn bran and stored in a glass container. Thus, the increase in ethyl carbamate during storage can be related to the type of yeast used in the production of the beverage. All the samples analyzed in the present work were produced from natural yeast with corn bran and using only

Table 1. Physicochemical composition of sugarcane brandy samples stored in stainless steel vats from 1996 to 2016.

Sample/Year	Alcohol conc.[1]	Dry matter[2]	Volatile acidity[3]	Furfural[3]	Aldehydes[3]	Esters[3]	Higher alcolhols[3,4]	Congeners[5]
A/1996	39.51±0.16^{i}	1.37± 0.00^{a}	83.95±0.35^{c}	5.03±0.03^{a}	7.16±0.61^{h}	30.19±0.13^{e}	146.58±0.94^{g}	272.91±1.73^{f}
B/1998	41.97±0.05^{h}	1.07± 0.01^{b}	143.69±0.16^{a}	2.32±0.05^{b}	10.97±0.22^{e}	55.48±3.55^{b}	131.24±0.45^{h}	343.69±2.90^{c}
C/1999	43.13±0.27^{g}	0.24±0.01^{g}	72.25±6.24^{d}	1.95±0.07^{c}	15.93±0.48^{c}	58.08±0.71^{d}	146.47±1.49^{g}	294.67±18.62^{f}
D/2000	36.68±0.09^{j}	0.61±0.01^{d}	19.19±1.71^{h}	1.35±0.02^{d}	3.91±0.25^{j}	6.19±0.81^{g}	167.86±6.02^{e}	198.49±5.76^{g}
E/2001	41.84±0.03^{h}	0.30±0.00^{f}	69.66±1.46^{e}	1.14±0.01^{d}	8.66±0.01^{g}	31.22±0.83^{e}	147.49±0.24^{g}	258.17±2.46^{f}
F/2002	44.47±0.01^{f}	0.49±0.01^{e}	20.34±0.00^{h}	0.94±0.06^{e}	12.90±0.55^{d}	21.07±1.36^{f}	137.93±0.58^{h}	193.18±1.42^{g}
G/2003	41.71±0.04^{h}	0.83±0.00^{c}	59.03±1.49^{f}	1.81±0.14^{c}	6.15±0.80^{i}	34.03±0.81^{e}	167.74±0.42^{e}	268.76±2.95^{f}
H/2008	44.46±0.02^{f}	0.12±0.00^{i}	73.47±3.70^{d}	0.63±0.02^{e}	11.20±0.01^{e}	42.79±2.06^{d}	158.64±0.42^{f}	286.73±4.42^{e}
I/2010	48.38±0.08^{c}	0.16±0.04^{h}	68.55±0.11^{e}	1.57±0.03^{c}	10.45±0.38^{e}	53.99±0.65^{b}	157.05± 1.75^{f}	291.61±1.35^{e}
J/2011	47.10±0.03^{d}	0.17±0.03^{h}	113.73±1.19^{b}	0.80±0.71^{e}	8.17±0.35^{g}	75.96±2.51^{a}	173.25± 2.05^{d}	371.90±4.64^{b}
K/2012	49.31±0.01^{b}	0.23±0.01^{g}	76.42±0.01^{d}	1.35±0.16^{d}	19.28±0.32^{b}	47.22±0.70^{c}	135.91± 8.80^{h}	280.18±8.18^{e}
L/2014	46.76±0.03^{e}	0.12±0.00^{i}	32.24±0.02^{g}	0.52±0.01^{e}	15.33±0.53^{c}	31.57±0.76^{e}	184.32± 1.88^{c}	263.98±2.72^{f}
M/2015	49.49±0.01^{b}	0.08±0.01^{i}	77.16±3.28^{d}	1.41±0.13^{d}	19.97±0.49^{b}	47.04±2.52^{c}	162.43± 1.82^{f}	308.02±5.00^{d}
N1/2016	47.17± 0.01^{d}	0.10±0.00^{i}	79.90±0.01^{c}	0.71±0.07^{e}	39.67±0.70^{a}	52.37±1.28^{b}	264.79± 2.96^{a}	437.44±2.43^{a}
N2/2016	51.38±0.01^{a}	0.10±0.00^{i}	30.48±0.34^{g}	0.64±0.05^{e}	9.37±0.05^{f}	29.58±0.06^{e}	216.11±4.28^{b}	286.18±4.63^{e}
Limit*	**38-54**	**6**	**150**	**5**	**30**	**200**	**360**	**200 - 650**

Results are expressed as mean ± standard deviation; means followed by the same letter in the column do not differ significantly from each other by the Scott-Knott test at the 5% probability level. [1]% (v/v). [2]mg/L. [3]mg/100 mL of anhydrous alcohol. [4]Higher alcohols = sum of alcohols (propyl + isoamyl + isobutyl). [5]Volatile acidity + esters + aldehydes + furfural + higher alcohols. *BRAZIL, 2005a.

Table 2. Concentrations of contaminants in sugarcane spirits stored in stainless steel vats from 1996 to 2016.

Sample/Year	Butan-1-ol^{1}	Butan-2-ol^{1}	Methanol^{1}	Ethyl Carbamate^{2}	Copper^{3}
A/1996	4.07±0.08^{a}	2.21±0.00^{b}	1.92±0.01^{b}	51.47±0.93^{a}	1.96±0.00^{h}
B/1998	2.99±0.05^{c}	1.97±0.00^{d}	1.80±0.02^{c}	33.66±0.01^{d}	2.86±0.03^{e}
C/1999	2.52±0.10^{d}	2.13±0.04^{c}	1.74±0.01^{d}	37.74± 1.08^{c}	1.41±0.03^{j}
D/2000	1.37±0.02^{g}	2.02± 0.01^{d}	2.86±0.16^{a}	31.22± 0.02^{e}	0.16±0.01^{l}
E/2001	2.39±0.01^{e}	1.89± 0.02^{e}	1.81±0.02^{c}	31.44± 0.52^{e}	3.47±0.08^{b}
F/2002	2.18±0.03^{f}	2.12± 0.02^{c}	1.71±0.01^{e}	37.16± 0.87^{c}	1.05±0.01^{k}
G/2003	3.54±0.05^{b}	2.14± 0.00^{c}	1.84±0.02^{c}	41.15± 0.35^{b}	2.34±0.03^{g}
H/2008	0.74±0.01^{j}	ND	1.68±0.01^{e}	34.45± 0.70^{d}	3.07±0.00^{d}
I/2010	1.14±0.05^{h}	1.91± 0.01^{e}	1.57±0.02^{g}	18.36± 0.10^{g}	2.74±0.04^{f}
J/2011	0.79±0,04^{j}	2.27± 0.06^{b}	1.74±0.02^{d}	13.90± 0.64^{h}	2.76±0.01^{f}
K/2012	0.80±0.02^{j}	1.47± 0.03^{f}	1.51±0.02^{h}	14.50± 1.15^{h}	3.10±0.02^{d}
L/2014	0.77±0.03^{j}	ND	1.87±0.02^{b}	29.36± 2.03^{e}	1.44±0.03^{j}
M/2015	0.72±0.02^{j}	2.08±0.00^{c}	1.64±0.01^{f}	21.78± 1.02^{f}	3.21±0.02^{c}
N1/2016	0.65±0.01^{j}	4.76±0.02^{a}	ND	<LQ$^{3\ j}$	4.09±0.03^{a}
N2/2016	0.92±0.01^{i}	1.22± 0.01^{g}	ND	9.24±0.06^{i}	1.96±0.03^{i}
Limit*	**3**	**10**	**20**	**210****	**5**

Results are expressed as mean ± standard deviation; means followed by the same letter in the column do not differ significantly from one another by the Scott-Knott test at the 5% probability level. ^{1}mg/100 mL of anhydrous alcohol. ^{2}µg/L. ^{3}mg/L. ND: Not Detected. LQ: Limit of Quantification. *BRAZIL (2005a). **BRAZIL (2014).

the heart fraction during storage. Zacaroni et al. (2015) evaluated the effect of light on the concentration of ethyl carbamate in cachaça stored for up to six months in the presence and absence of light. They observed that its concentration decreased in some samples that were kept in the dark from the second month of storage, and some of these samples were below the limit of quantification. The authors concluded that light significantly influenced the presence of this organic contaminant in the matrix.

Metallic contaminants

None of the samples contained copper concentrations that exceeded that allowed by current legislation. This fact means that the producer effectively cleaned his distillation equipment and kept the copper concentrations in his beverages at safe levels. In addition to copper, beverage samples were also analyzed for the presence of cadmium, chromium, lead, zinc, and iron by atomic absorption. However, only zinc was detected at concentrations of 0.3 mg/L in sample B/1998; 0.9 mg/L in sample E/2001; 0.4 mg/L in sample G/2003 and 0.5 mg/L in sample H/2008. The WHO advises that the concentration of zinc should not exceed 5 mg/L in drinking water because higher concentrations can cause depression of the immune system and gastric symptoms (Pinto et al., 2005). Thus, one can conclude that there was no metal contamination in beverages stored in stainless steel vats. Tábua (2020) investigated distilled beverages from different regions of Mozambique and found contamination in the Mozambican samples by metals such as copper (ND to 34.83 mg/L), lead (600 to 3660 mg/L), zinc (ND to 23.01 mg/L) and iron (ND at 0.04 mg/L). The author attributes the presence of these metals to failures in hygiene in the process and equipment and to the characteristics of the soil and the raw material used. However, the presence of cadmium or chromium was not detected in any of the samples from that African country.

Materials and Methods

Production and collection of samples

The sugarcane spirit samples were produced at Engenho Boa Vista, located in the city of Coronel Xavier Chaves, Minas Gerais, Brazil. The collection was achieved in 2016; 15 samples were acquired from the 1996 to 2016 harvests of beverages stored since the year of production in cylindrical stainless steel tanks (type 304) with a capacity of 2000 liters, separated according to the batch of production (Tables 3).

Physicochemical analysis

The samples were sent to the Laboratory of Aguardente Quality Analysis at the Chemistry Department of the Federal University of Lavras (UFLA). The physical-chemical analyzes were performed in triplicate in accordance with the specifications established by Normative Instruction no. 24, of September 8, 2005, of MAPA (Brasil, 2005b). Dry extract, volatile acidity, alcoholic concentration, aldehydes, esters, furfural, methanol, higher alcohols, ethyl carbamate and copper were quantified in the samples.

Quantification of metals

Lead, iron, zinc, cadmium and chromium metals were analyzed by Flame Atomic Absorption Spectroscopy (Varian Spectr AA 110 Atomic Absorption). Analytical curves were constructed using standard solutions at the following concentrations: iron (1.0, 2.0, 4.0, 8.0, 10.0 mg/L); zinc (0.25, 0.50, 1.00, 1.50, 3.00 mg/L), lead (2.0, 4.0, 6.0, 8.0 mg/L), chromium (0.5, 1.0, 2.5, 5 mg/L), cadmium (0.5, 1.0, 2.0, 3.0 mg/L). Readings were performed at wavelengths: 248.3 nm for iron, 213.9 nm for zinc and 217.0 nm for lead, using a hollow cathode lamp. All readings were performed in triplicate (Brasil, 2005b).

Table 3. Samples of sugarcane spirit stored in stainless steel vats from 1996 to 2016 harvests.

Sample	Harvest	Storage Time (Years)
A	1996	20
B	1998	19
C	1999	18
D	2000	17
E	2001	16
F	2002	15
G	2003	14
H	2008	9
I	2010	7
J	2011	6
K	2012	5
L	2014	3
M	2015	2
N1	2016	1
N2	2016	1

Chromatographic analysis

The quantification of methanol and higher alcohols (3-methylbutan-1-ol, 2-methylpropan-1-ol, propan-1-ol, butan-1-ol and butan-2-ol) was performed by gas chromatography equipped with a flame Ionization detector (GC-FID) using the method proposed by Vilela et al., (2007). The quantification of ethyl carbamate was performed by previous sample derivation and analyzed by HPLC according to methods proposed by Anjos et al., (2011), Machado et al., (2013) and Santiago et al., (2017).

Determination of glycerol

The glycerol concentration in the beverage was determined by the colorimetric method proposed by Bortoletto et al., (2016). The UV/Vis absorbance of the samples was measured at 410 nm in a spectrophotometer (Shimadzu UV-1601 PC). Concentrations were calculated with the aid of an analytical curve previously constructed with standard glycerol solutions at concentrations of 7.5, 15, 22.5, 30, 45, 60 and 90 mg/L.

Statistical analysis

A completely randomized design (CRD) in split-plot space was used. Data from the analysis results were normalized by the Box-Cox method (Box and Cox, 1964) and then evaluated by the Scott-Knott test (Scott and Kanott, 1974), with 95% confidence, with the aid of the R statistical software (R Core Team, 2017) to determine whether there was a significant difference between the samples for each parameter analyzed. Analyzes were performed in triplicate, and the results were expressed as means ± standard deviations.

Conclusion

Among the 15 samples of sugarcane spirit stored for 20 years in stainless steel vats, the results for five samples were outside the limits required by legislation (A/1996, D/2000, F/2002, G/2003 and N1/2016); that is, they were not suitable for commercialization and consumption. In some cases, this result can be associated with a long period of storage, as in the case of higher alcohols, whose concentration decreased during storage, thereby contributing to a decrease in the concentration of congeners in some samples. Despite the fact that stainless steel is an iron-based metal alloy, the presence of this metal was not detected in the samples, even after 20 years of storage. The metals cadmium, chromium and lead were also not detected. Zinc was detected in eight of the 15 samples, but at concentrations below 1.0 mg/L. Copper was found in legally permitted concentrations. These results indicate that the storage of the beverage in stainless steel containers for a period of up to 20 years modified the physicochemical characteristics of the beverage, but it did not affect the quality of the beverage or pose risks to the consumer regarding metal contamination, and it can be used to store the sugarcane distillate.

Conflict of interest

The authors declare that no conflict of interests exists.

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